Note that no proper claim "67" was in this case previously. Claim 66 had been erroneously designated to be "67". An early action on the merits is solicited.

Respectfully submitted,

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Attached: Claims 67-100

PENDING CLAIMS DOCKET 100/150C1 U.S.S.N. 07/205,419 AUGUST 22, 1990

- 67. A process for producing an Ig molecule or an immunologically functional Ig fragment comprising at least the variable domains of the Ig heavy and light chains, in a single host cell, comprising the steps of:
 - (i) transforming said single host cell with a first DNA sequence encoding at least the variable domain of the Ig heavy chain and a second DNA sequence encoding at least the variable domain of the Ig light chain, and
 - (ii) independently expressing said first DNA sequence and said second DNA sequence so that said Ig heavy and light chains are produced as separate molecules in said transformed single host cell.
- 68. The process according to claim 67 wherein said first and second DNA sequences are present in different vectors.
- 69. The process according to claim 67 wherein said first and second DNA sequences are present in a single vector.
- 70. A process according to claim 68 wherein the vector is a plasmid.
- 71. A process according to claim 70 wherein the plasmid is pBR322.
- 72. A process according to claim 67 wherein the host cell is a bacterium or yeast.
- 73. A process according to claim 72 wherein the host cell is *E. coli* or *S. cerevisiae*.

- 74. A process according to claim 73 wherein the host cell is $E.\ coli$ strain X1776.
- 75. A process according to claim 67 wherein the Ig heavy and light chains are expressed in the host cell and secreted therefrom as an immunologically functional Ig molecule or Ig fragment.
- 76. A process according to claim 67 wherein the Ig heavy and light chains are produced in insoluble form and are solubilized and allowed to refold in solution to form an immunologically functional Ig molecule or Ig fragment.
- 77. A process according to claim 67 wherein the DNA sequences code for the complete Ig heavy and light chains.
- 78. A process according to claim 67 wherein said first or said second DNA sequence further encodes at least one constant domain, wherein the constant domain is derived from the same source as the variable domain to which it is attached.
- 79. A process according to claim 67 wherein said first or said second DNA sequence further encodes at least one constant domain, wherein the constant domain is derived from a species or class different from that from which the variable domain to which it is attached is derived.
- 80. A process according to claim 67 wherein said first and second DNA sequences are derived from one or more monoclonal antibody producing hybridomas.
- 81. A vector comprising a first DNA sequence encoding at least a variable domain of an Ig heavy chain and a second DNA sequence encoding at least

a variable domain of an Ig light chain wherein said first DNA sequence and said second DNA sequence are located in said vector at different insertion sites.

- 82. A vector according to claim 81 which is a plasmid.
- 83. A host cell transformed with a vector according to claim 81.
- 84. A transformed host cell comprising at least two vectors, at least one of said vectors comprising a DNA sequence encoding at least a variable domain of an Ig heavy chain and at least another one of said vectors comprising a DNA sequence encoding at least the variable domain of an Ig light chain.
- 85. The process of claim 67 wherein the host cell is a mammalian cell.
- 86. The transformed host cell of claim 84 wherein the host cell is a mammalian cell.

87. A method comprising

- a) preparing a DNA sequence consisting essentially of DNA encoding an immunoglobulin selected from the group consisting of an immunoglobulin heavy chain, light chain, and Fab region, said immunoglobulin having specificity for a particular known antigen;
- inserting the DNA sequence of step a) into a replicable expression vector operably linked to a suitable promoter;
- c) transforming a prokaryotic or eukaryotic microbial host cell culture with the vector step b);
- d) culturing the host cell; and
- e) recovering the immunoglobulin from the host cell culture, said immunoglobulin being capable of binding to a known antigen.

- 88. The method of claim 87 wherein the heavy or light chain are the heavy or light chains of anti-CEA antibody.
- 89. The method of claim 87 wherein the heavy chain is of the gamma family.
- 90. The method of claim 87 wherein the light chain is of the kappa family.
- 91. The method of claim 87 wherein the vector contains DNA encoding both a heavy chain and a light chain.
- 92. The method of claim 87 wherein the host cell is \underline{E} . coli or yeast.
- 93. The method of claim 92 wherein the heavy chain, light chain or Fab region is deposited within the cells as insoluble particles.
- 94. The method of claim 93 wherein the heavy or light chains are recovered from the particles by cell lysis followed by solubilization in denaturant.
- 95. The method of claim 87 wherein the heavy or light chain is secreted into the medium.
- 96. The method of claim 87 wherein the host cell is a gram negative bacterium and the heavy or light chain is secreted into the periplasmic space of the host cell bacterium.
- 97. The method of claim 87 further comprising recovering both heavy and light chain and reconstituting light chain and heavy chain to form an immunoglobulin having specific affinity for a particular known antigen.
- 98. The insoluble particles of heavy chain, light chain, or Fab region produced by method of claim 93.

- 99. A replicable expression vector comprising DNA operably linked to a promoter compatible with a suitable procaryotic or eukaryotic microbial host cell, said DNA consisting essentially of DNA encoding an immunoglobulin heavy chain, light chain or Fab region having specificity for a particular known antigen.
- 100. Recombinant host cells transformed with the vector of claim 99.